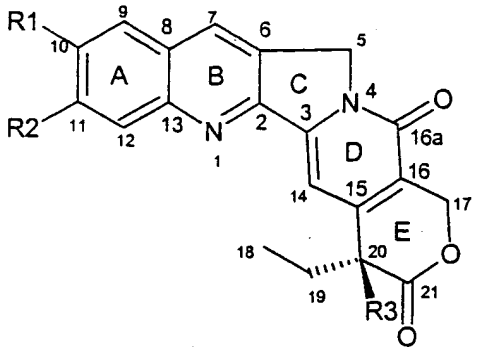
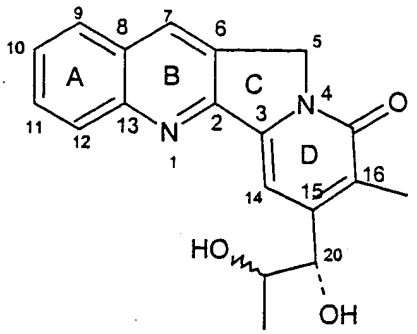


1 R_1=R_2=H, R_3=OH
2 R_1=R_3=OH, R_2=H
3 R_1=OCH_3, R_2=H,R_3=OH
4 R_1=H, R_2=R_3=OH
5 R_1=H, R_2=OCH_3, R_3=OH
6 R_1=R_2=R_3=H
7 R_1=R_2=H, R_3=$O(CH_2)_4CH_3$
8 R_1=OCH_3, R_2=H, R_3=$O(CH_2)_4CH_3$



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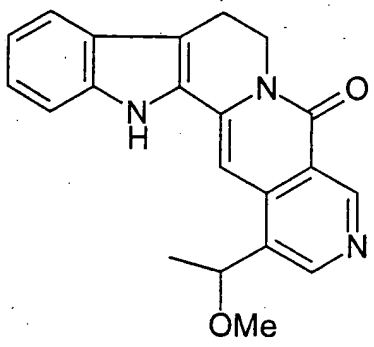

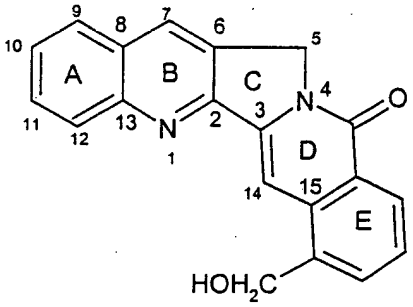

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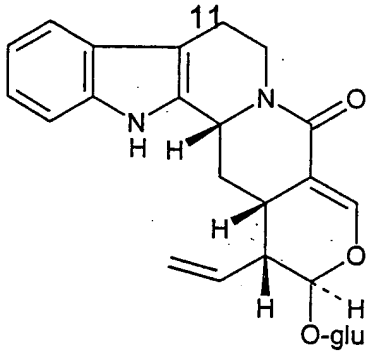


12

Diagrams of the chemical structures of major natural camptothecin and its analogs in Camptotheca acuminata: camptothecin (1), 10-hydroxycamptothecin (2), 10 methoxycamptothecin (3), 11-hydroxycamptothecin (4), 11-methoxycamptothecin (5), 20-deoxycamptothecin (6), 20-hexanoylcamptothecin (7), 20-hexanoyl-10-methoxycamptothecin (8), 22-hydroxyacuminatine (9), 19-hydroxymappicine (10), 19-O-methylangustoline (11), and vincoside-lactam (12).

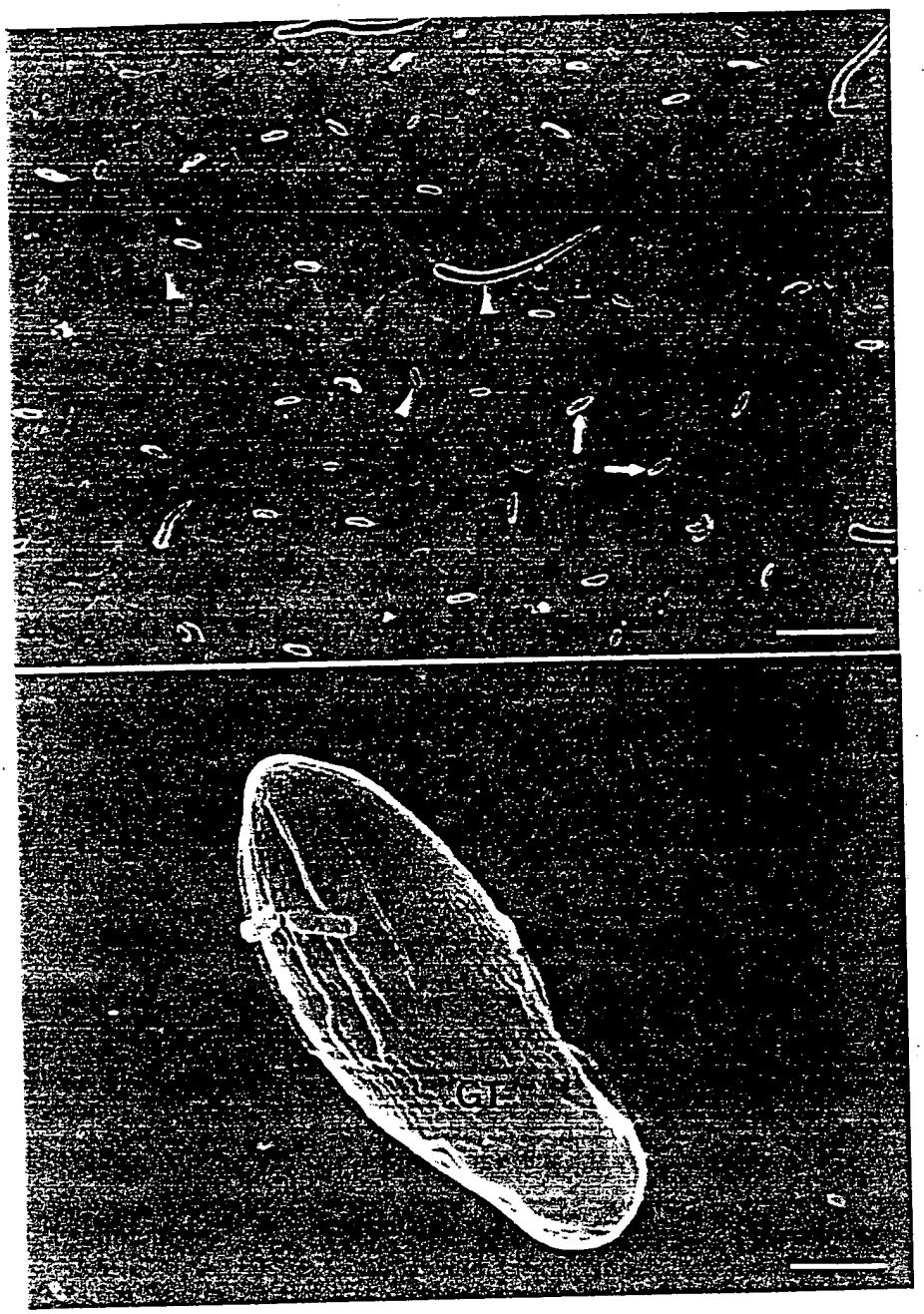
Fig. 1.

		Young Tissue	Intermediate Tissue	Old Tissue
Leaf	Definition	<1 week old	1-4 week old	>4 week old
	CPT % (fw)	0.05140 ± 0.00869	0.022450 ± 0.00135	0.01018 ± 0.00169
Stem	Definition	<4 week old	= 2 year old	= 5 year old
	CPT % (fw)	0.01063 ± 0.00313	0.00795 ± 0.00036	0.00648 ± 0.00078
Stem Wood	Definition	<1 year old	= 2 year old	= 5 year old
	CPT % (fw)	0.00662 ± 0.00014	0.00309 ± 0.00007	0.00566 ± 0.00027
Stem Pith	Definition	<1 year old	-----	-----
	CPT % (fw)	0.01433 ± 0.00053	-----	-----
Stem Bark	Definition	<1 year old	= 2 year old	= 5 years old
	CPT % (fw)	0.01380 ± 0.00257	0.01960 ± 0.00054	0.01010 ± 0.00313
Flower/Fruit	Definition	<1 week old (flower)	= 8 week old (fruit)	= 16 week old (fruit)
	CPT % (fw)	0.02276 ± 0.00280	0.01127 ± 0.00039	0.05058 ± 0.00294
Root	Definition	<4 weeks	-----	> 4 weeks
	CPT % (fw)	0.00171 ± 0.00005	-----	0.00526 ± 0.00105

Note: Shiyou Li, et al. unpublished.

CPT distribution in different tissues of <u>Camptotheca acuminata</u> (Seed source: SFA 94-03; leaf, stem, and root materials were collected in May, wood and bark samples were collected in August and fruit samples were collected in June, August, and October, respectively) (mean ± s.d.) (on the basis of fresh weight).

Fig. 2



a. [Top] Scanning electron micrograph of surface view of lower leaf epidermis of *Camptotheca Lowreyana* 'Katie'. Scale Bar = 100 μm.

b. [Bottom] Scanning electron micrograph of mature glandular trichome (GT) on lower leaf surface of *Camptotheca Lowreyana* 'Katie'. Scale Bar - 5μm.

Fig. 3

Species/Variety	Average Glandular trichome length (μm)	Average Glandular trichome width (μm)	Average Glandular trichome Density (μm)	Young Leaves CPT Concentration (% ± s.d.)	Old Leaves CPT Concentration (% ± s.d.)
C. acuminata	34.87 ± 3.92	13.64 ± 1.97	46.80 ± 5.26	0.05822 ± 0.01654	0.01607 ± 0.00204
C. yunnanensis	35.20 ± 2.70	13.72 ± 1.27	27.10 ± 11.40	0.05443 ± 0.01499	0.1308 ± 0.00217
C. lowreyana	46.49 ± 4.51	10.94 ± 1.42	81.50 ± 6.50	0.08423 ± 0.01541	0.02021 ± 0.0321
C. lowreyana 'Katie'	42.46 ± 3.34	14.64 ± 1.68	65.50 ± 10.41	0.10641 ± 0.01612	0.02174 ± 0.00167
C. lowreyana 'Hicksii'	56.32 ± 6.72	14.02 ± 1.87	69.20 ± 14.45	0.12284 ± 0.01089	0.02632 ± 0.00278

Note: Shiyou Li, et al. unpublished.

Glandular trichome size and density on lower leaf surfaces and CPT concentration in leaves of Camptotheca (on the basis of fresh weight.)

Fig. 4

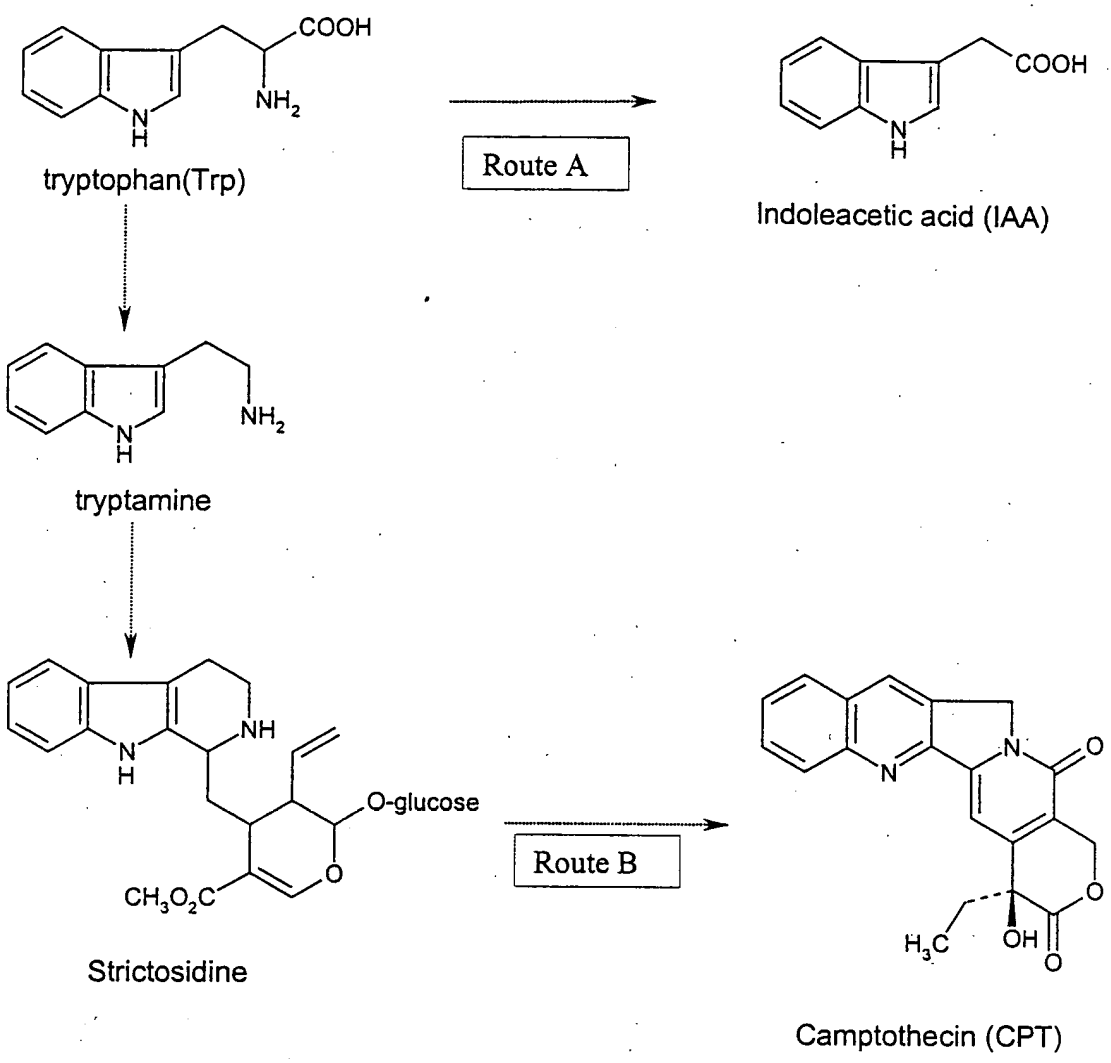
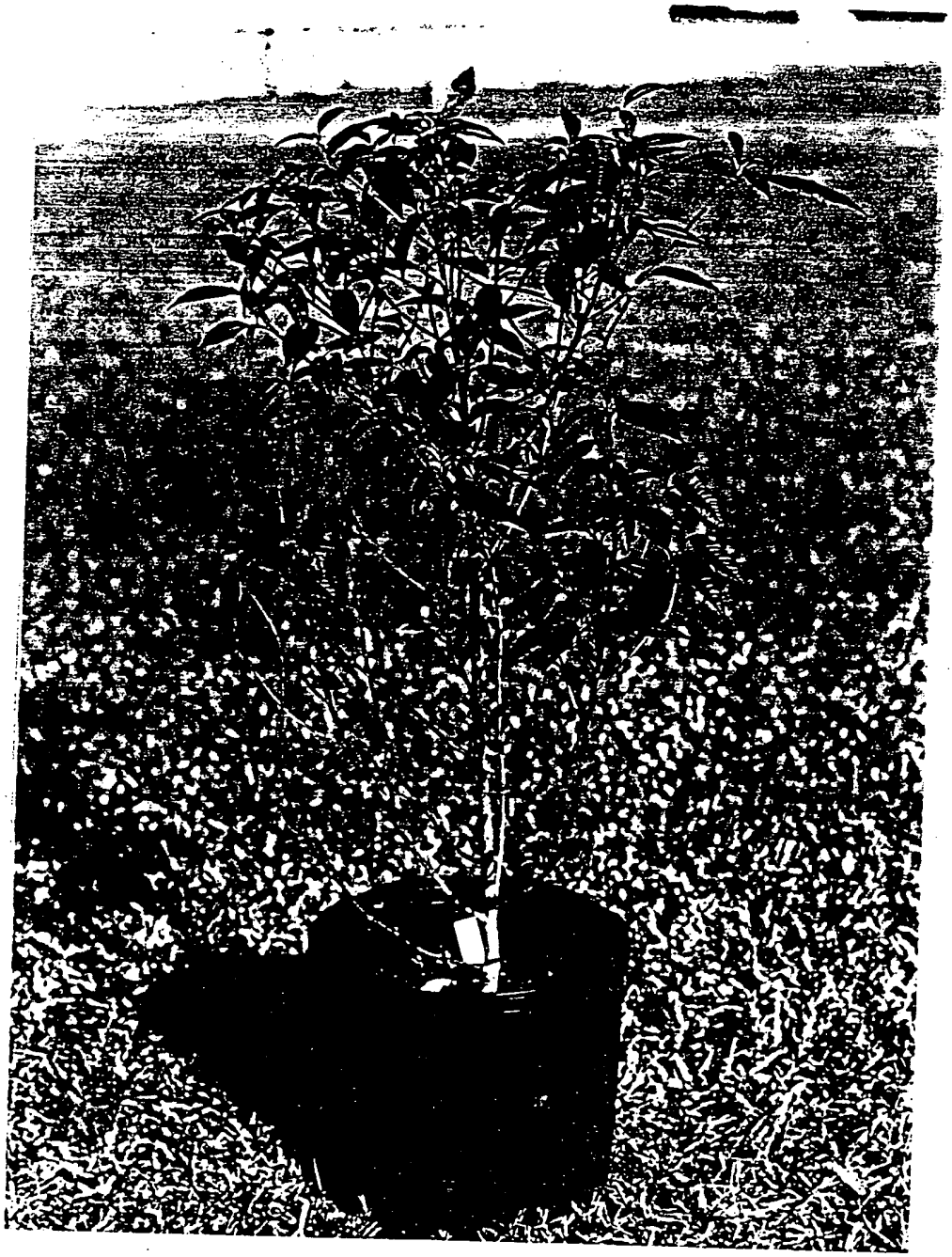


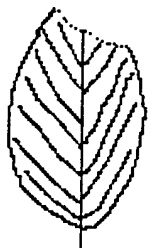
Diagram of two biosynthetic pathways showing tryptophan (TRP) as a biosynthetic precursor for both indoleacetic acid (Route A for stimulating growth) and camptothecin (Route B for inhibiting growth).

Fig. 5



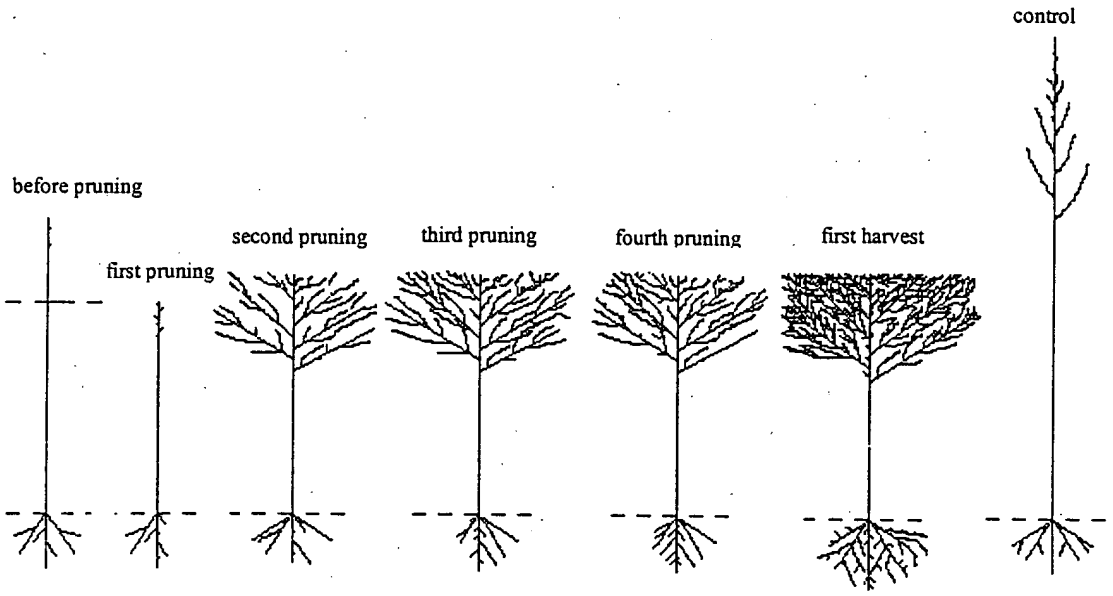
Photograph of Camptotheca lowreyana 'Katie'.

Fig. 6



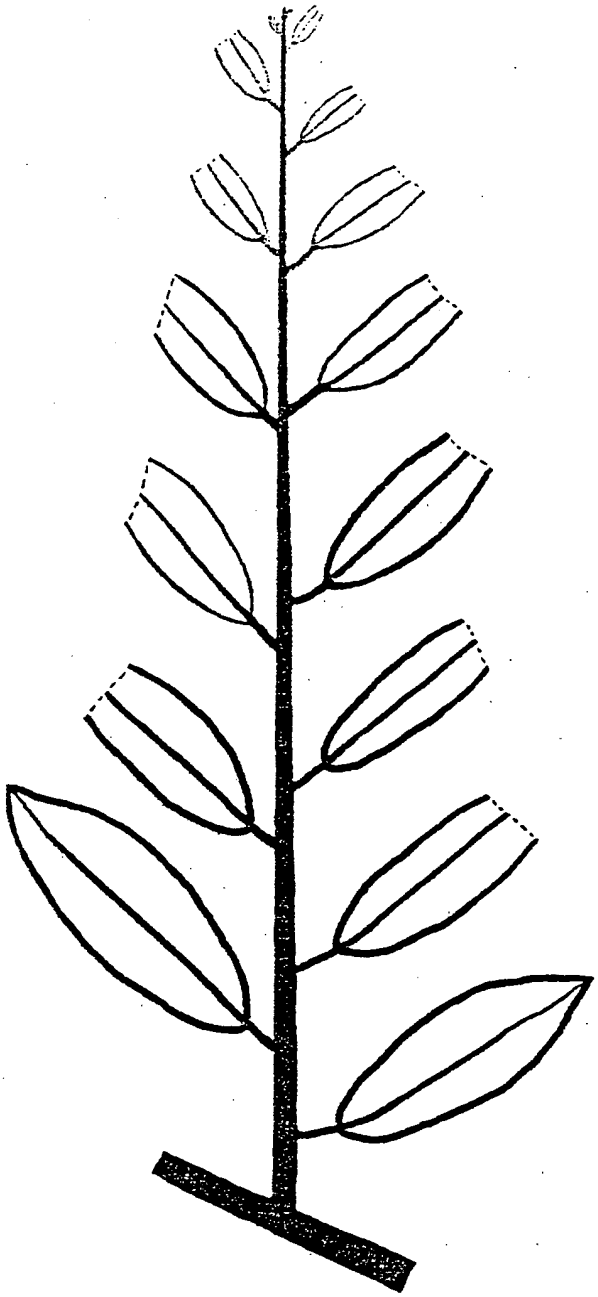
Drawing of a Camptotheca leaf after leaf-tip pinching.

Fig. 7



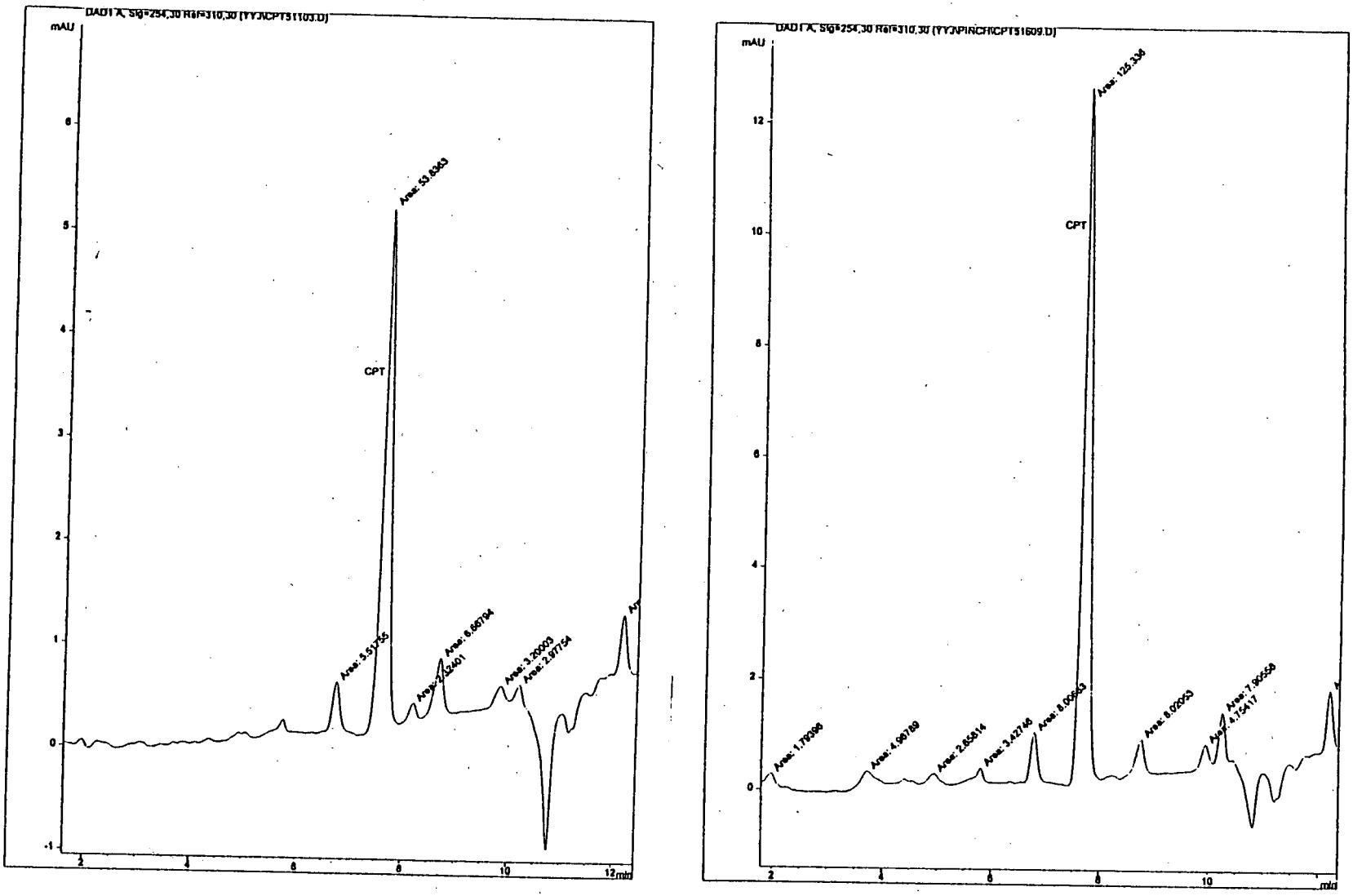
Drawing of a Camptotheca seedling T-pruning treatments and control.

Fig. 8



Drawing of the leaf-tip pinching technique as applied in Camptotheca.

Fig. 9

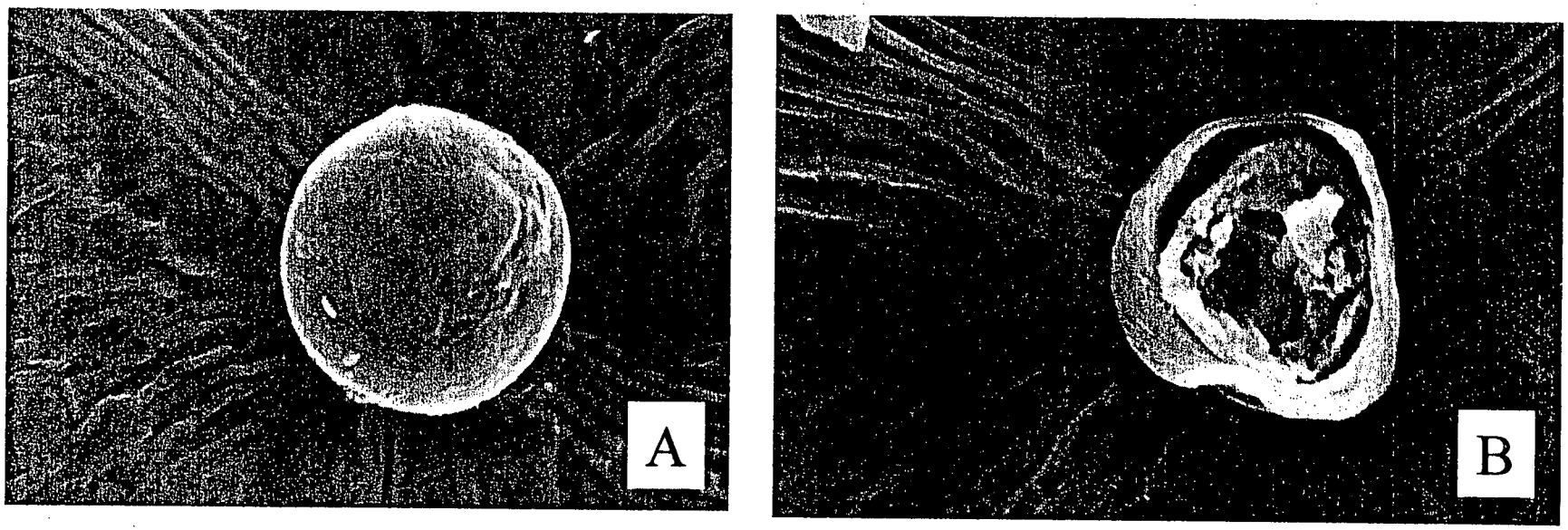


Picture of HPLC profiles showing the induction of CPT and its analogs in Camptotheca acuminata by pinching (a: control, b: pinching treatment).

a b

Fig. 10

Trichome Treatment



Before Treatment

After Treatment

Scanning electron micrograph of glandular trichome on upper leaf surface of Camptotheca acuminata.

Trichome on upper leaf surface of *Camptotheca acuminata*

Fig. 11

	Control (cm)	Treatment I (30 cm)	Treatment II (40 cm)
Before treatment (March 25, 1997)	52.61 ± 5.44	52.15 ± 3.57	50.72 ± 6.12
After treatment (March 25, 1997)	52.61 ± 5.44	30.00 ± 0.00	40.00 ± 0.00
Net Growth (March 25-July 12, 1997)	40.10 ± 8.86 a	39.59 ± 9.73 a	32.93 ± 7.82 b
Net Growth (July 12-Sept. 13, 1997)	19.52 ± 11.39 a	31.87 ± 8.41 b	32.00 ± 9.52 b

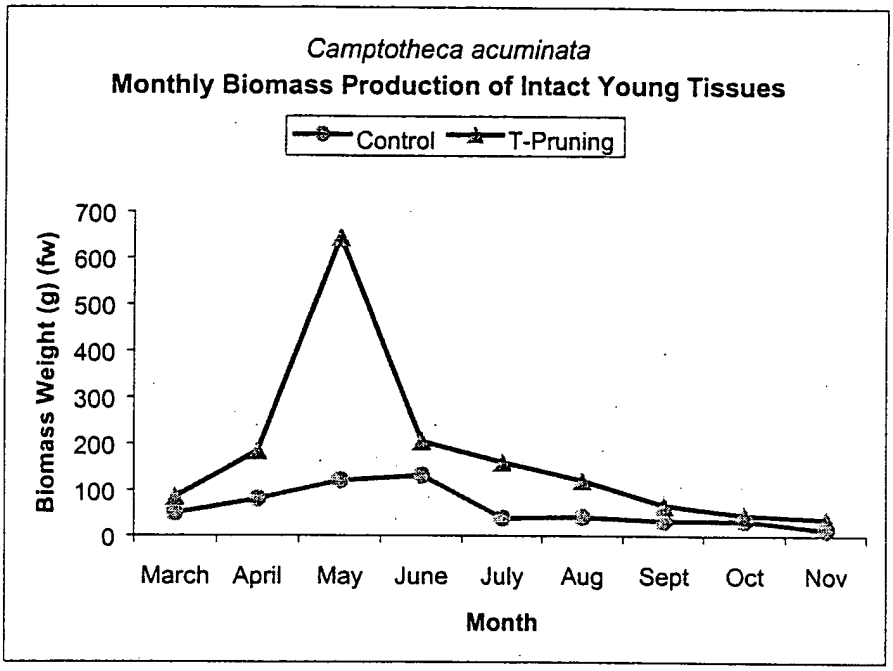
Mean height growth of plants with different T-pruning treatments (mean ± s.d.) (means with the same letter are not significantly different at α=0.05).

Fig. 12

	Control	Treatment I (30 cm)	Treatment II (40 cm)
Before treatment (March 25, 1997)	1.03 ± 0.17	1.04 ± 0.27	1.03 ± 0.17
After treatment (March 25, 1997)	1.03 ± 0.17	1.00 ± 0.00	1.00 ± 0.00
July 12, 1997	10.14± 3.06 a	10.21 ± 3.60	10.04 ± 3.16
Sept. 13, 1997	10.56 ± 3.34 a	17.00 ± 5.82 b	16.33 ± 5.21 b

Mean branch number of plants with different T-pruning treatments (mean ± s.d.) (means with the same letter are not significantly different at α=0.05).

Fig. 13



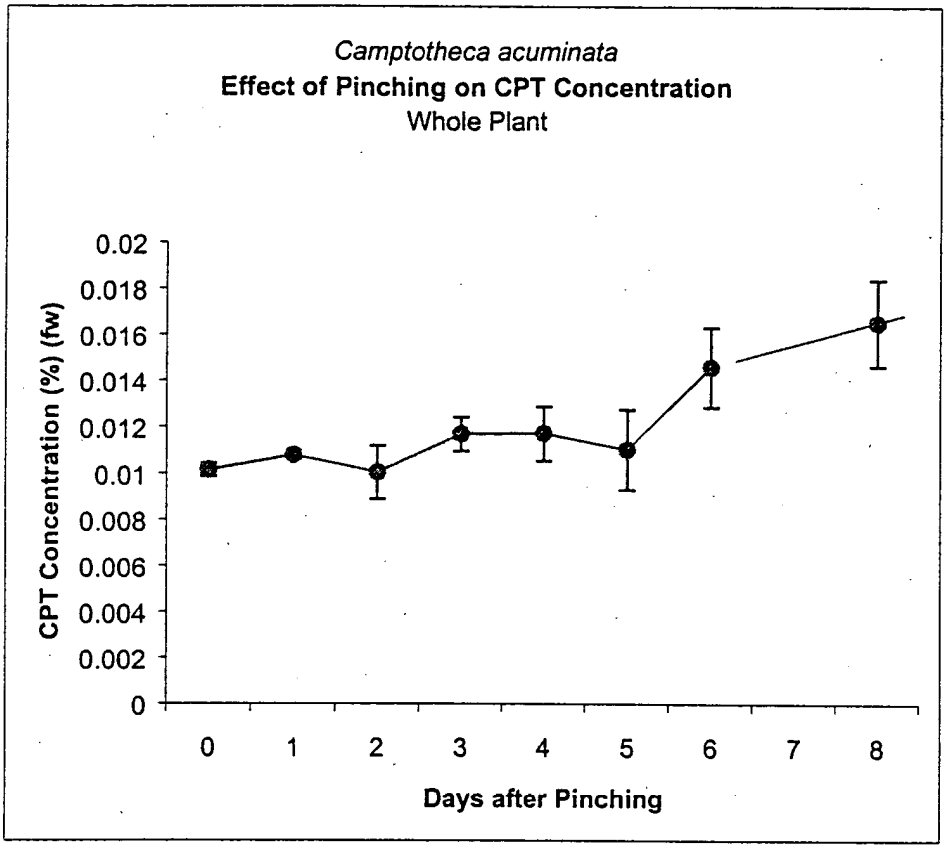
Graph of the monthly biomass production of intact young tissues with and without T-pruning

Fig. 14

	Control	Treatment I (30 cm)	Treatment II (40 cm)
CPT Content (%)	1.0164 ± 0.00141 a	0.0351 ± 0.0020 b	1.0437 ± 0.0037 c

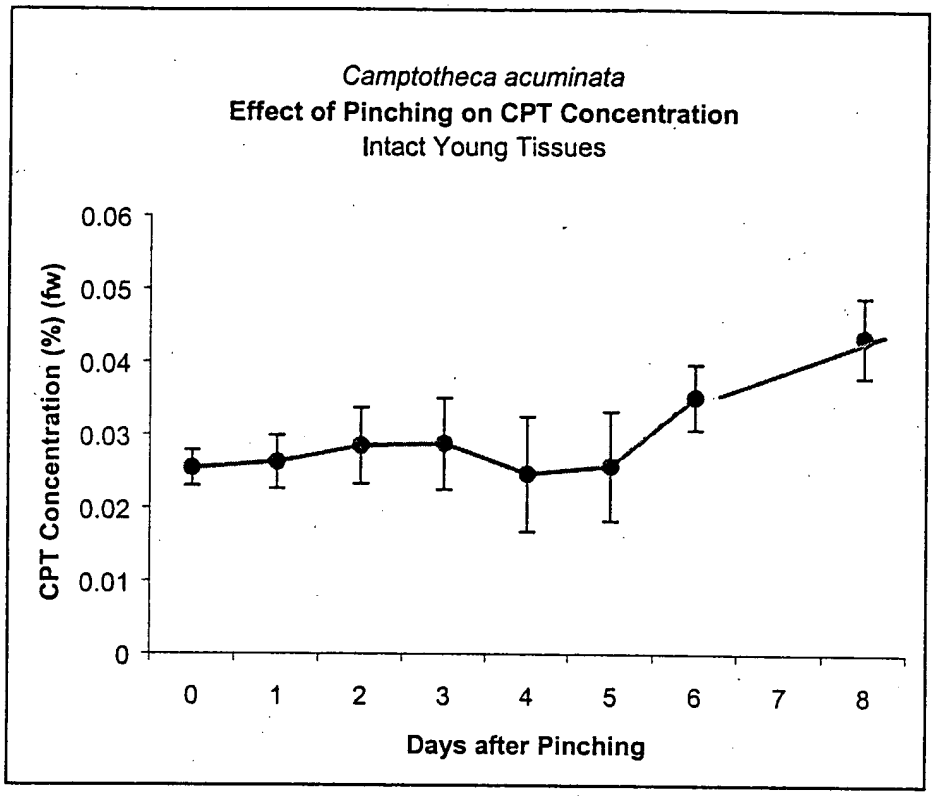
Effects of T-pruning treatments on CPT contents (%) of intact young tissues of Camptotheca acuminata (mean ± s.d.) (means with the same letter are not significantly different at α=0.05) (samples were collected on June 20, 1998) (fresh weight).

Fig. 15



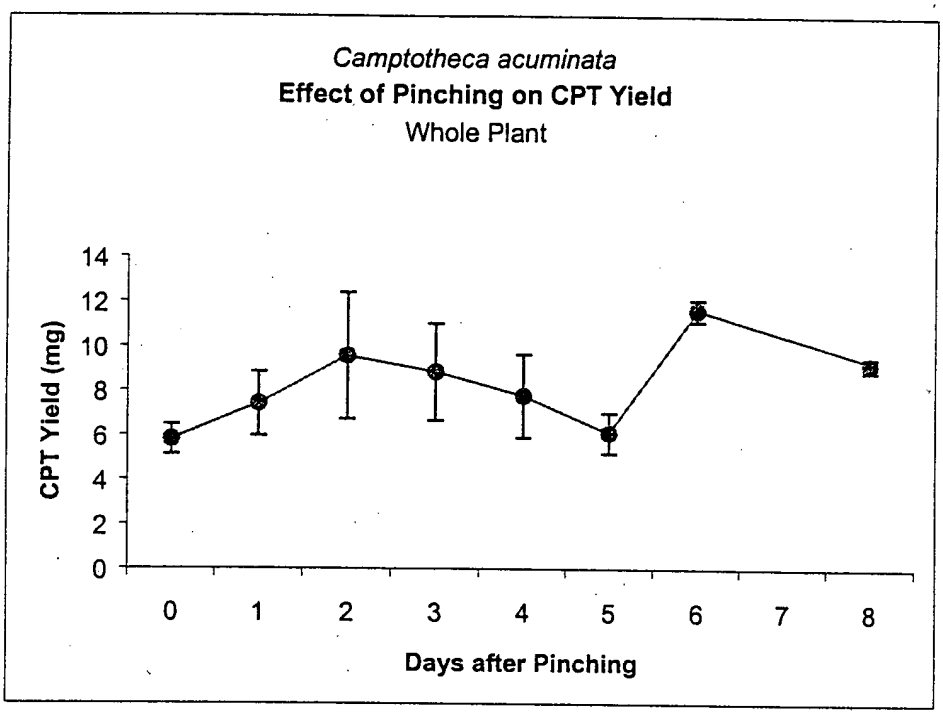
Graph of the effect of pinching on CPT concentration in the whole plant of Camptotheca acuminata.

Fig. 16a



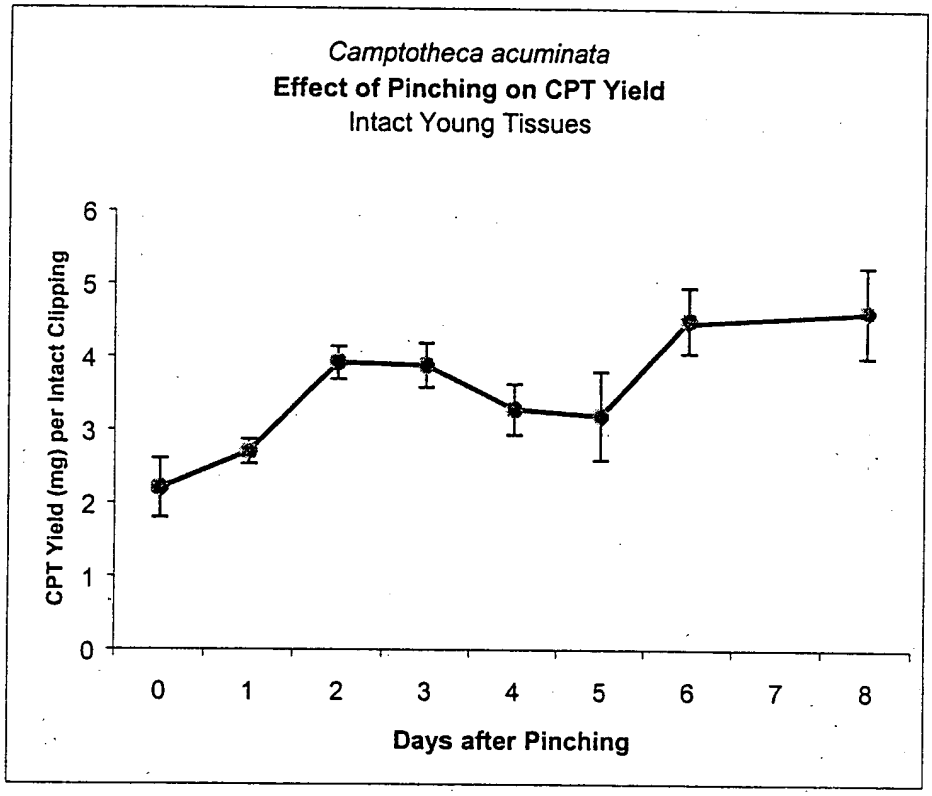
Graph of the effect of pinching on CPT concentration in intact young tissues of Camptotheca acuminata.

Fig. 16b



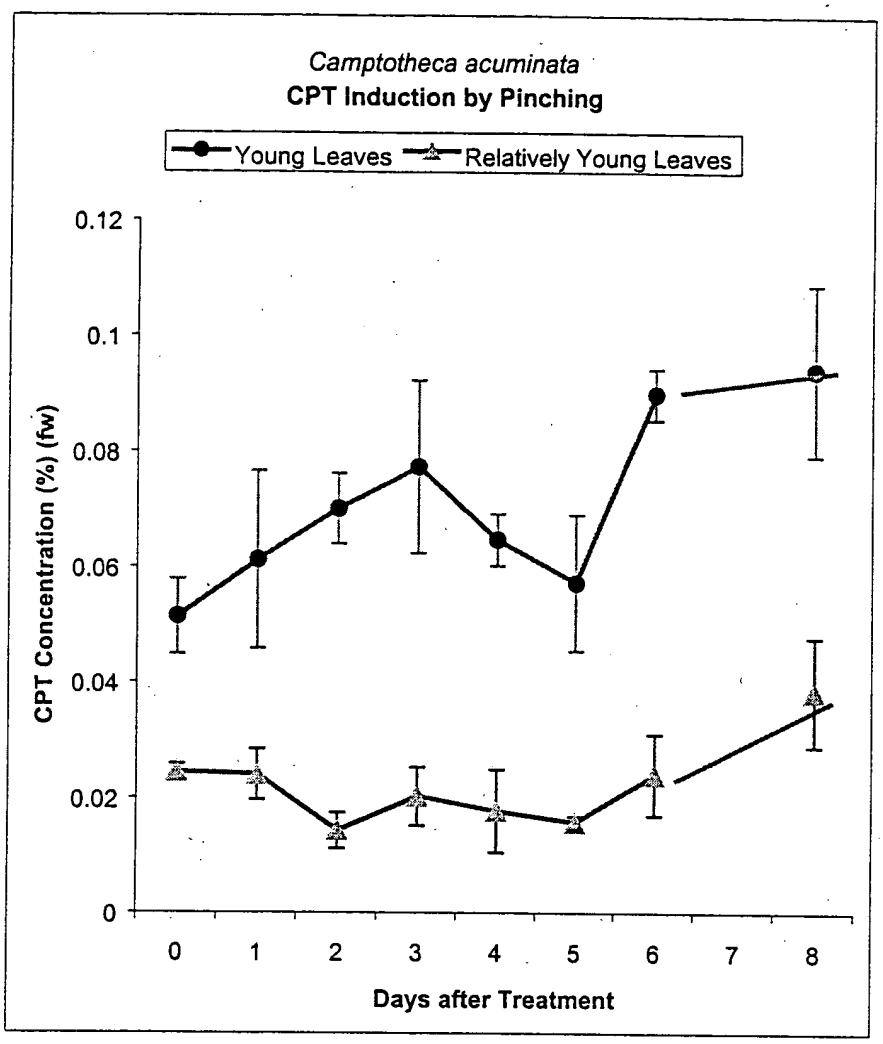
Graph of the effect of pinching on CPT yield on the whole plant in Camptotheca acuminata.

Fig. 16c



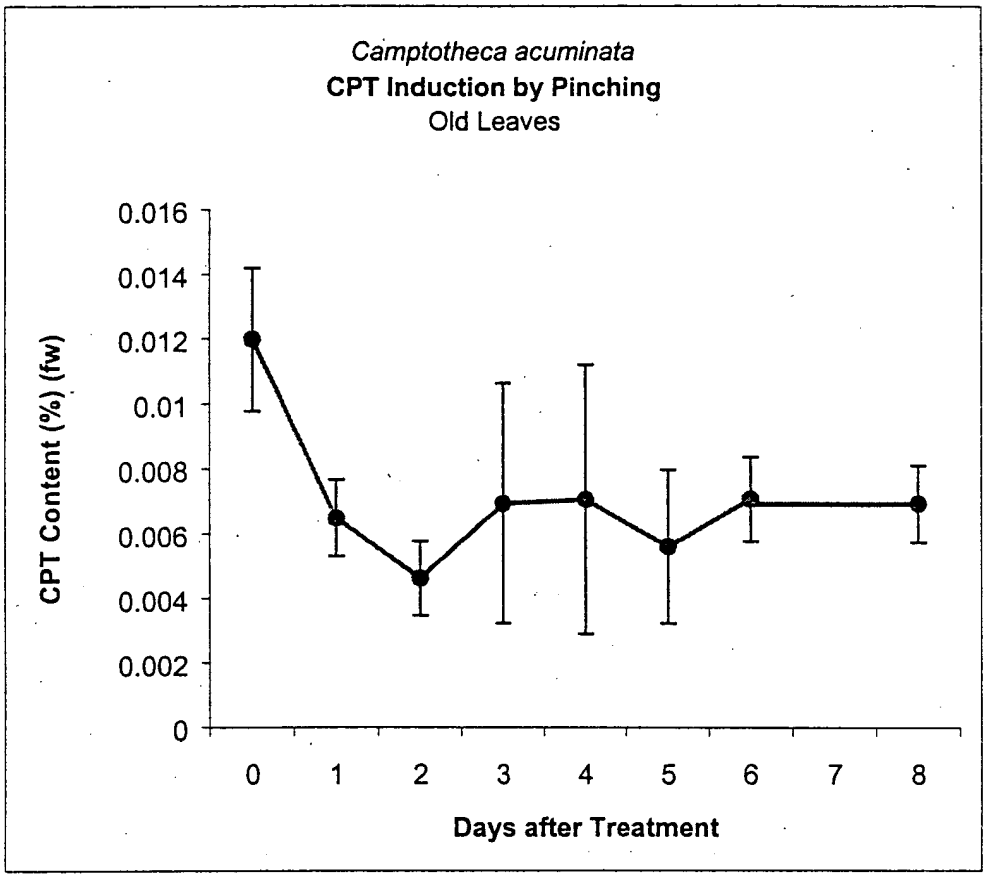
Graph of the effect of pinching on CPT yield on intact young tissues in Camptotheca acuminata.

Fig. 16d



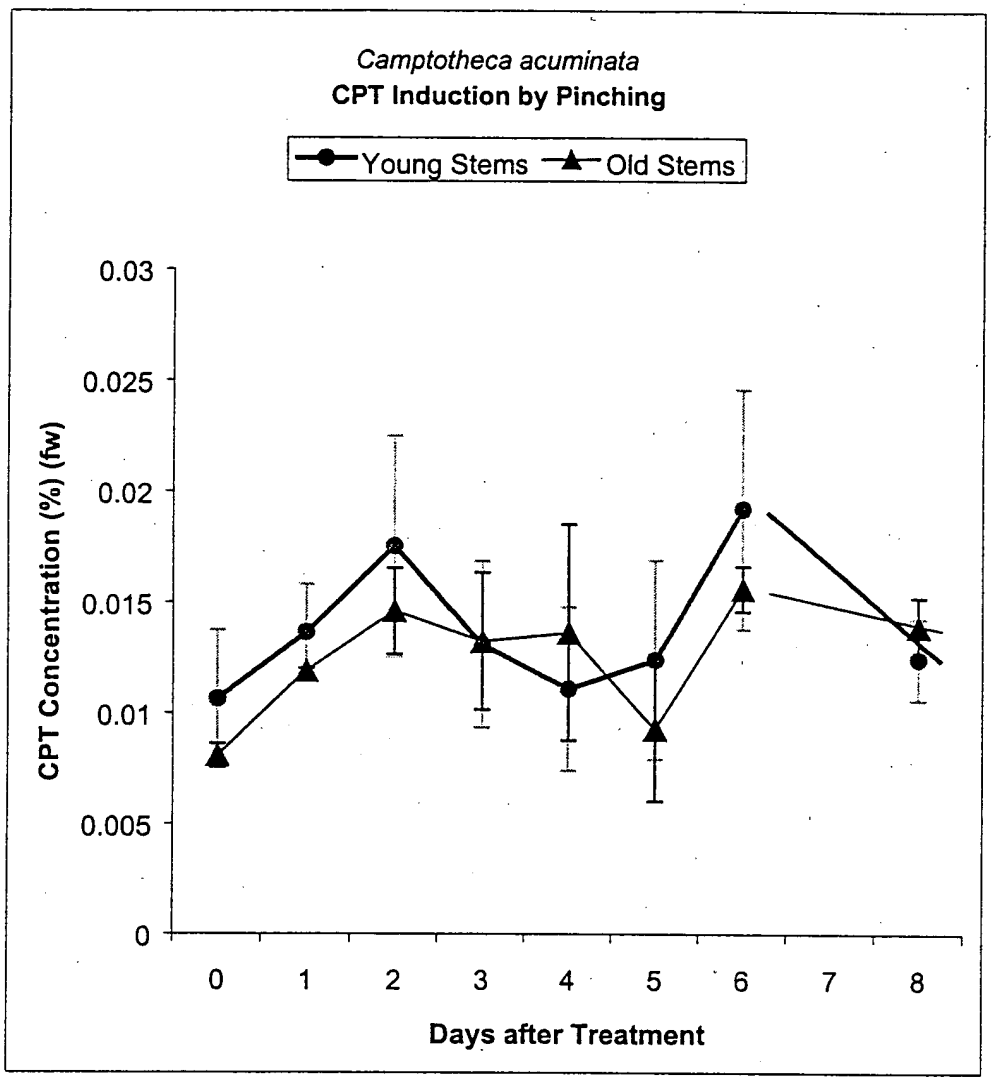
Graph of CPT induction by pinching in young leaves and relatively young leaves of Camptotheca acuminata.

Fig. 17a



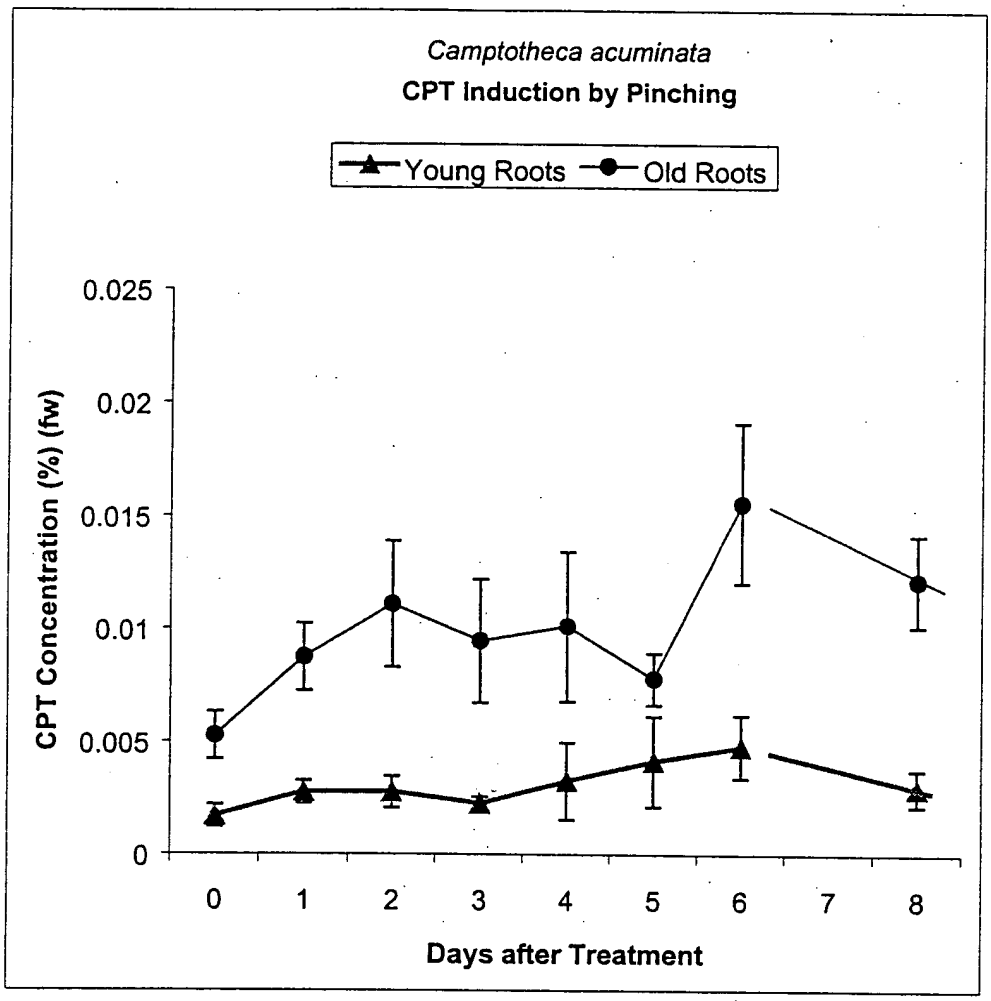
Graph of CPT induction by pinching old leaves of Camptotheca acuminata.

Fig. 17b



Graph of CPT induction by pinching in young stems and old stems in Camptotheca acuminata.

Fig. 17c



Graph of CPT induction by pinching in young roots and old roots in Camptotheca acuminata.

Fig. 17d

Treatment	CPT Concentration (%)
Pruning only	1.02506 ± 0.00389
Pruning + Pinching	0.03043 ± 0.00129

Effects of pinching treatments on CPT contents (%) of intact young tissues of Camptotheca acuminata under irrigation system (mean ± s.d.) (means with the same letter are not significantly different at α=0.05) (samples were collected on August 30, 2000) (fresh weight).

Fig. 18

Light Levels	Sample Size	Height	Living Branch Number
Full Sunlight	25	52.78 ± 13.08 a	1.12 ± 0.33 a
Shade	23	82.23 ± 14.51 b	2.26 ± 0.52 b

Mean growth of one-year-old seedlings grown under different light levels (mean ± s.d.) (means with the same letter are not significantly different at α=0.05) (data were collected on July 12, 1998.

Fig. 19

Light Levels	Sample Size	Height	Living Branch Number	Glandular Trichome Density (no./mm2)
Full Sunlight	19	377.89 ± 59.99	17.05 ± 5.08	52.16
Shade	18	110.61 ± 21.92 b	3.67 ± 1.46	78.23 b

Mean growth and glandular tricome density of three -year-old seedlings grown under different light levels (mean ± s.d.) (means with the same letter are not significantly different at α=0.05) (data were collected on July 15, 1998.

Fig. 20

Treatment	Biomass (g) (fw)	CPT Concentration (%) (fw)	CPT yield (mg)
Natural Dry Condition	74.60 ± 17.74	0.05041 ± 0.00940 a	37.2112 ± 9.8481 a
Under Irrigation	213.29 ± 39.15 b	0.02754 ± 0.00648 b	59.2765 ± 22.0071 a

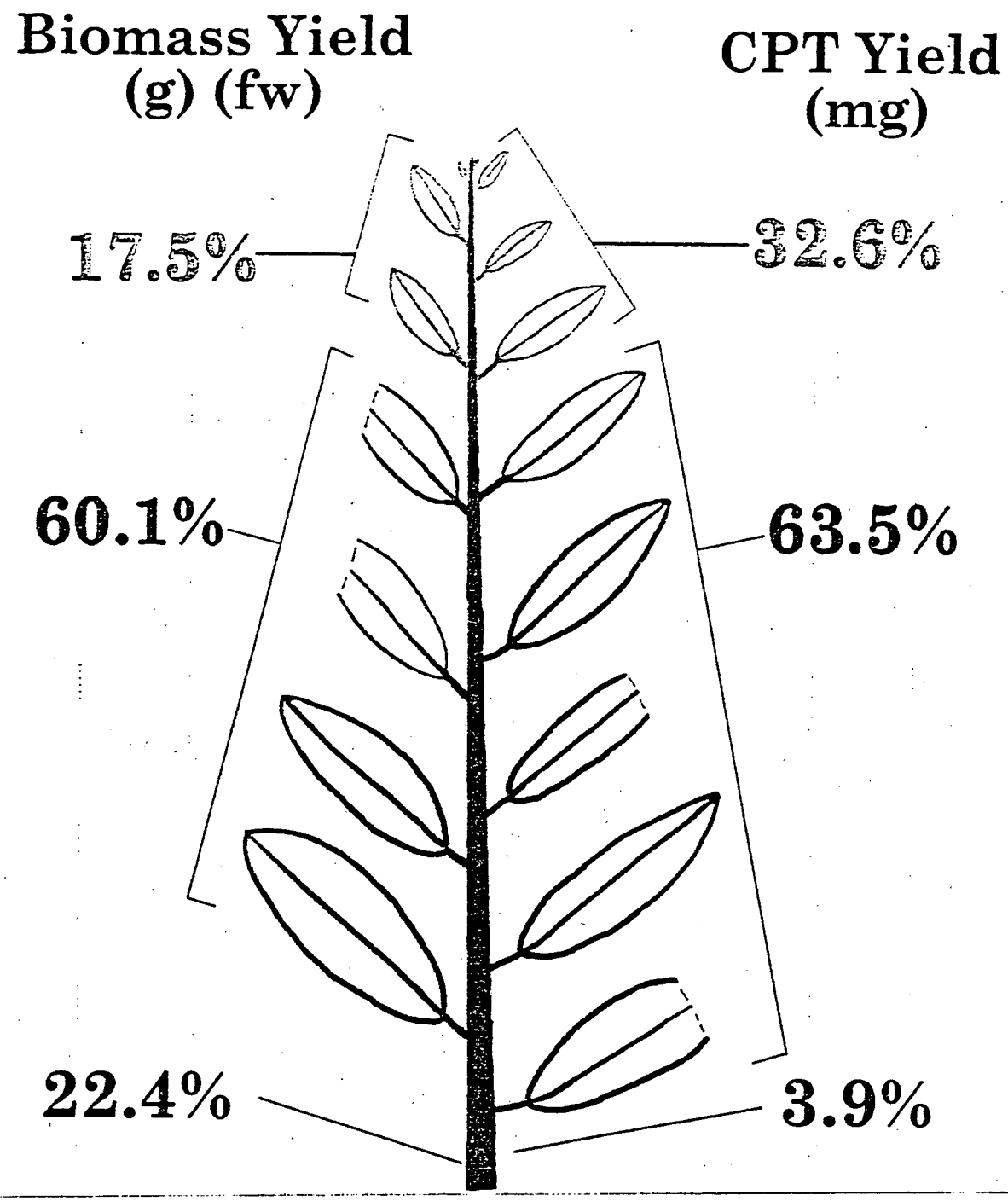
Production of biomass and CPT of intact young tissues under different water conditions (mean ± s.d.) (means with the same letter are not significantly different at α=0.05) (data were collected on August 30, 2000.

Fig. 21

	Young Leaves	Relatively Young Leaves	Young Stems	Intact Clipping
Biomass (g) (fw)	2.2485 ± 0.1039 a	7.7315 ± 0.1506 b	2.8950 ± 1.6334	12.8950 ± 1.3789
CPT Content (%) (fw)	0.0380 ± 0.0053 a	0.0214 ± 0.0031 b	0.0080 ± 00030 c	0.0203 ± 0.0008
CPT Yield (mg)	0.8504 ± 0.0806 a		0.1002 ± 0.1007 c	2.6054 ± 0.1804

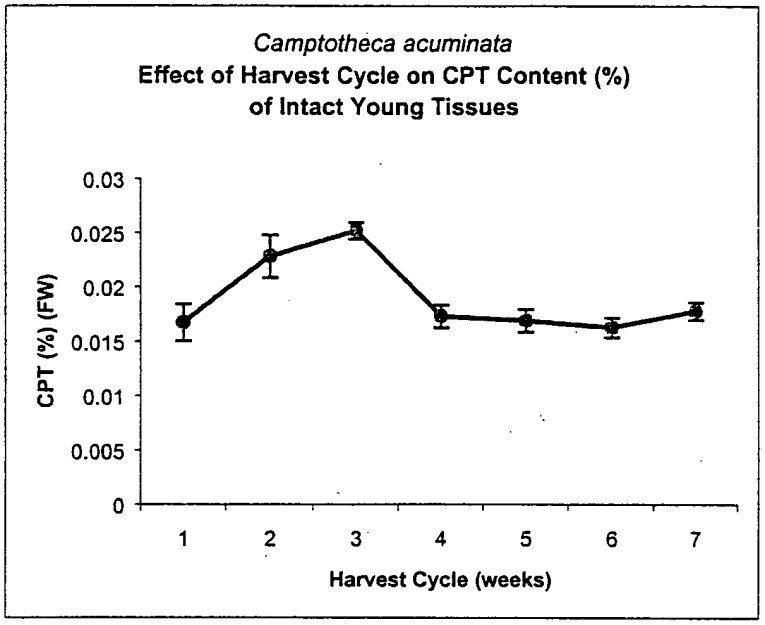
Distribution pattern of biomass, CPT content, and CPT yield in an intact clipping (mean ± s.d.) (means with the same letter are not significantly different at α=0.05) (Samples were collected on May 4, 2000).

Fig. 22



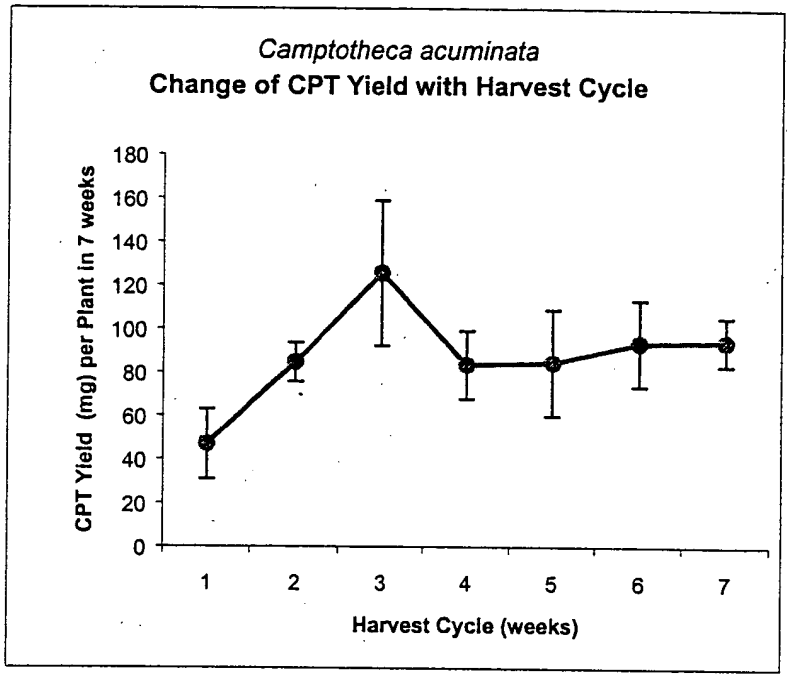
Total CPT Yield: 2.6 mg/Intact Clipping

Fig. 23



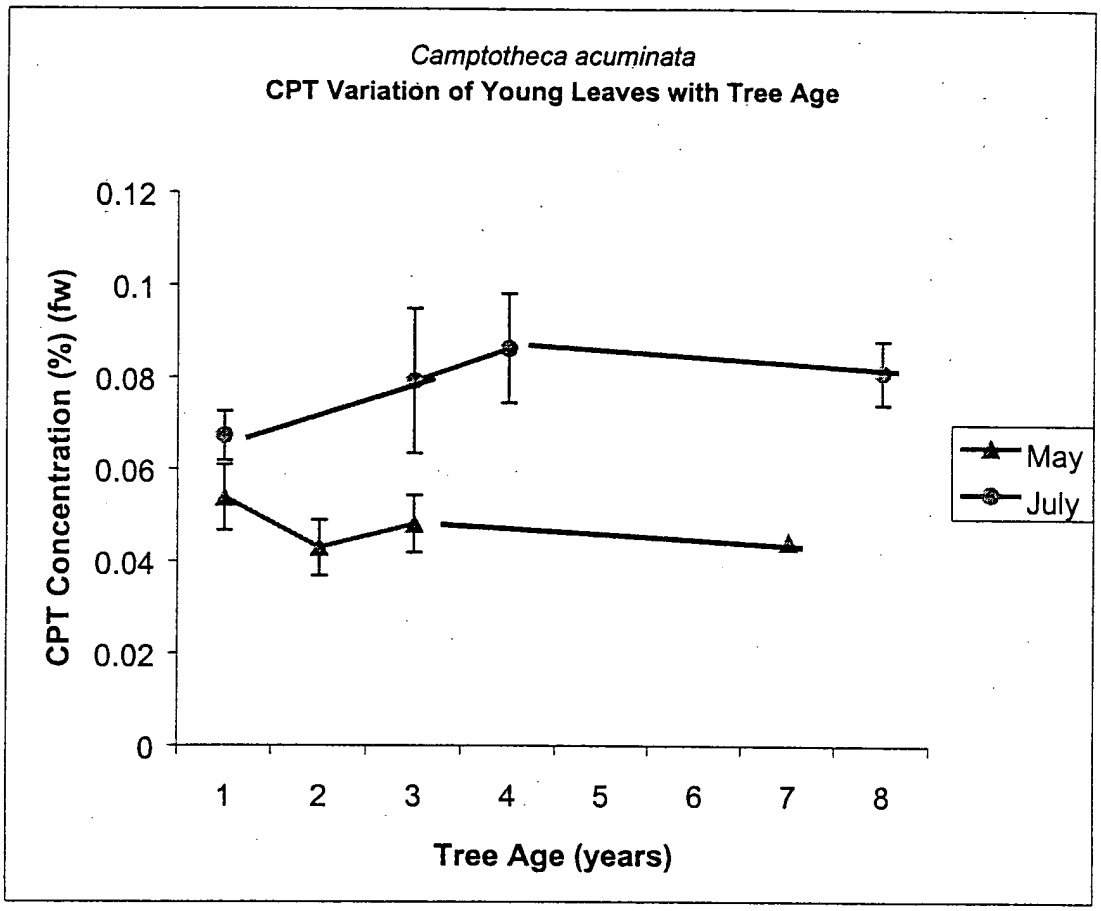
Graph of the effect of harvest cycle on CPT content of intact young tissues in Camptotheca acuminata.

Fig. 24a



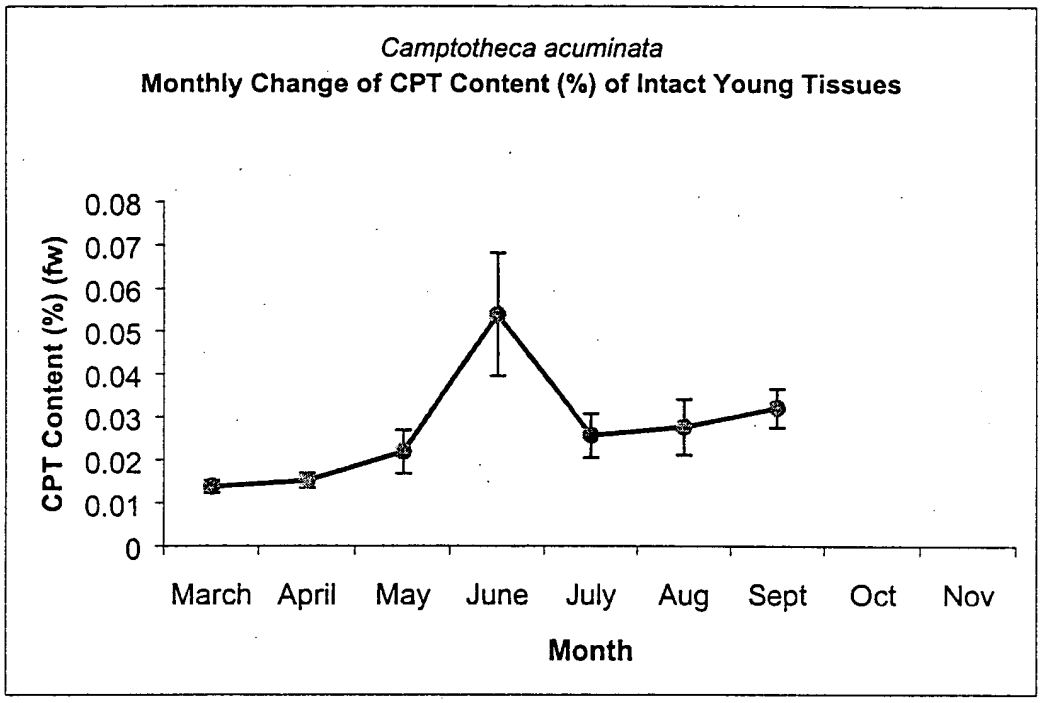
Graph of the effect of harvest cycle on CPT yield of intact young tissues in Camptotheca acuminata.

Fig. 24b



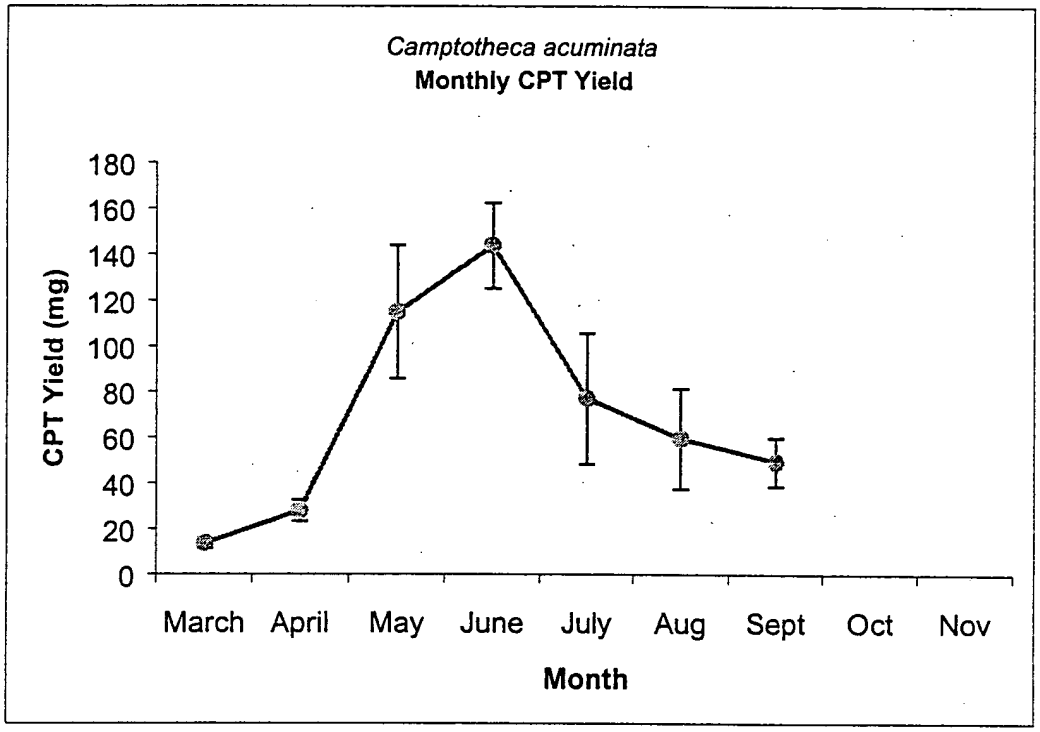
Graph of the variation in CPT concentration of young leaves with tree age in Camptotheca acuminata.

Fig. 25



Graph of the monthly change of CPT content of intact young tissues of Camptotheca acuminata.

Fig. 26a



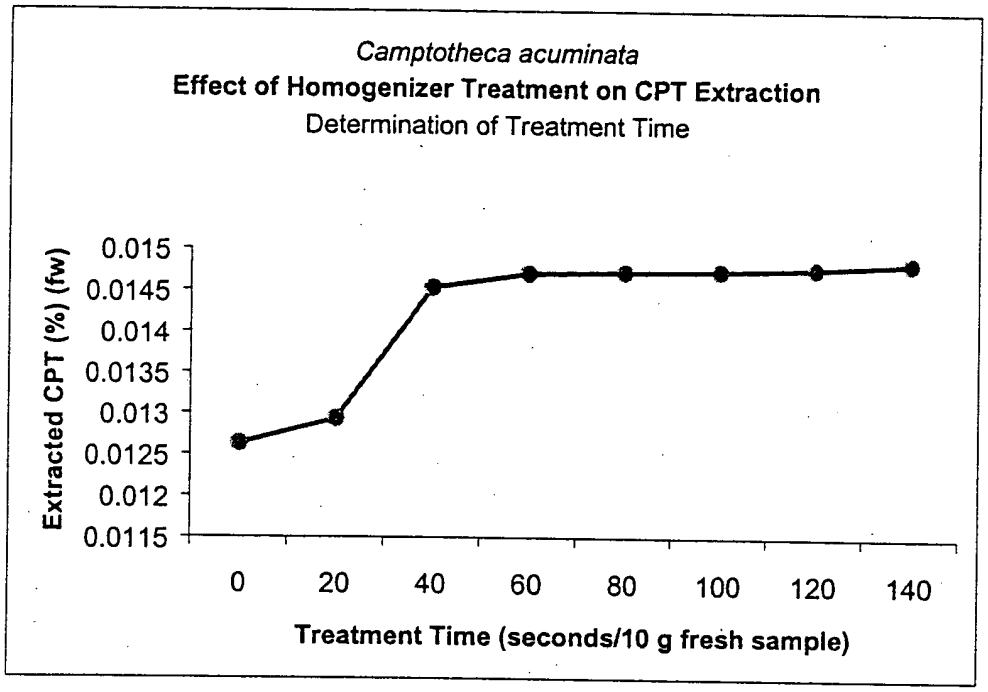
Graph of the monthly yield of CPT of intact young tissues of Camptotheca acuminata.

Fig. 26b

Preservation Method	CPT Content (% ± s.d.)
Fresh	0.03433 ± 0.0080
Freeze	0.03494 ± 0.0074
Vacuum-dry	0.03124 ± 0.00559
Air-dry	0.03015 ± 0.00797
Oven-dry (65°C)	0.02715 ± 0.0061

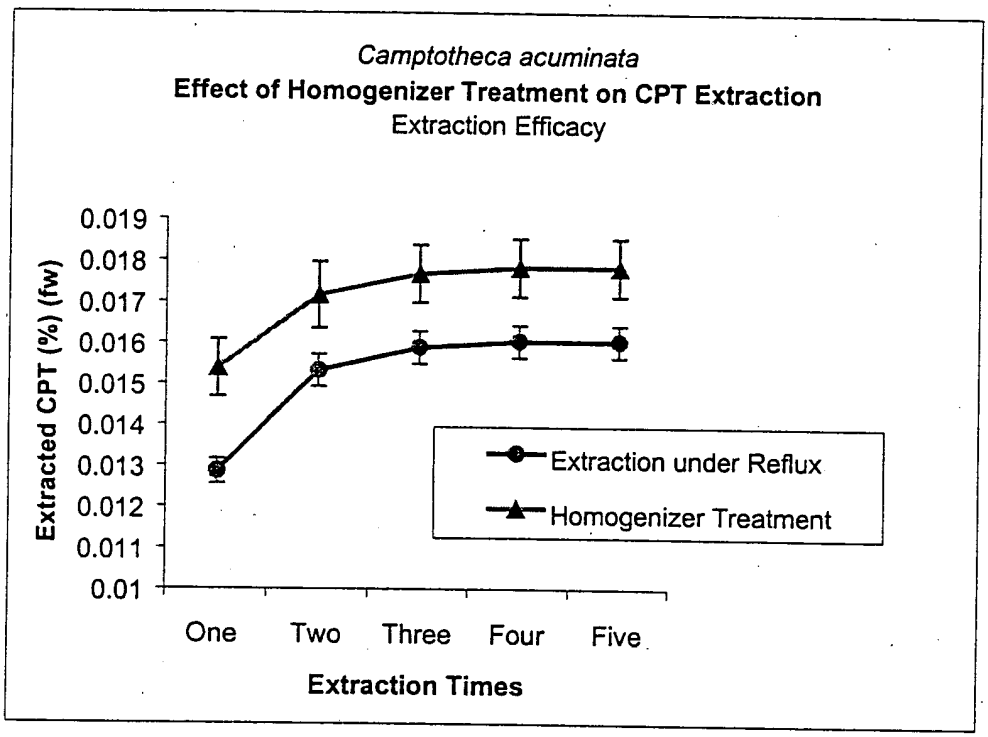
CPT Preservation of intact young tissues preserved by different methods (samples were collected on May 31, 2000) (6 replications, fresh weight).

Fig. 27



Graph of the effect of homogenizer treatment on CPT extraction in Camptotheca acuminata by duration of treatment time.

Fig.28a



Graph of the effect of homogenizer treatment on CPT extraction in Camptotheca acuminata by extraction efficacy.

Fig. 28b